AWARD NUMBER:
W81XWH-15-1-0385

TITLE:
Reprogramming the Metastatic Microenvironment to Combat Disease Recurrence

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REPORT DATE:
October 2016

TYPE OF REPORT:
Annual Report -1

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2016	Annual	30Sep2015 - 29Sep2016

4. TITLE AND SUBTITLE

Reprogramming the Metastatic Microenvironment to Combat Disease Recurrence

5a. CONTRACT NUMBER
W81XWH-15-1-0385

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Washington University
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT **This research aims to "eliminate the mortality associated with metastatic breast cancer". We will do so by: 1) testing a novel combination of clinically available therapeutic agents that modulate the metastatic tumor microenvironment for the prevention of metastatic disease and 2) using existing breast cancer patient samples to create a biomarker panel that combines disseminated tumor cell (DTC) classification and "immune-subtyping" to identify those patients most likely to benefit from this new treatment approach. In spite of recent advances in patient stratification and the use of targeted adjuvant therapies, 10- 20% of patients with invasive tumors will eventually develop metastatic disease1,2. These data indicate that for a significant percentage of patients, adjuvant therapies are ineffective at eliminating DTCs, which give rise to life threatening metastatic lesions. Therefore, the development of new clinical approaches that are effective at preventing metastatic breast cancer (BC) is of paramount importance. One promising approach is to "reprogram" the tissue microenvironments that provide "safe harbor" for DTCs during adjuvant therapy. Bone is one such metastatic "safe harbor." Sixty-seven percent of metastatic patients will develop disease in the bone3, which has been proposed to be a reservoir of malignant cells destined for subsequent metastatic relapse in the bone and visceral organs4. Interactions between DTCs and the bone microenvironment lead to pathological bone loss, which can stimulate tumor cell outgrowth. In addition to contributing to morbidity, this 'vicious cycle' also protects tumor cells from the cytotoxic effects of chemotherapy4. This protective microenvironment may also create an immune privileged site that prevents immune surveillance5 and effective immunotherapy. To better target bone metastasis with immunotherapy, we have been examining how T cell immune surveillance and antigen presentation affect metastatic relapse in the bone. In so doing, we discovered that inhibiting colony stimulating factor-1 receptor (CSF1R), which blocks pathologic activities of osteoclasts (OCs), serendipitously also increases antigen presentation by CD103+ dendritic cells (DCs).**

15. SUBJECT TERMS

Nothing listed

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	8	19b. TELEPHONE NUMBER *(include area code)*

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1. Introduction.

This research aims to "eliminate the mortality associated with metastatic breast cancer". We will do so by: **1)** testing a novel combination of clinically available therapeutic agents that modulate the metastatic tumor microenvironment for the prevention of metastatic disease and **2)** using existing breast cancer patient samples to create a biomarker panel that combines disseminated tumor cell (DTC) classification and "immune-subtyping" to identify those patients most likely to benefit from this new treatment approach.

In spite of recent advances in patient stratification and the use of targeted adjuvant therapies, 10-20% of patients with invasive tumors will eventually develop metastatic disease[1,2]. These data indicate that for a significant percentage of patients, adjuvant therapies are ineffective at eliminating DTCs, which give rise to life threatening metastatic lesions. Therefore, the development of new clinical approaches that are effective at preventing metastatic breast cancer (BC) is of paramount importance. One promising approach is to "reprogram" the tissue microenvironments that provide "safe harbor" for DTCs during adjuvant therapy. Bone is one such metastatic "safe harbor." Sixty-seven percent of metastatic patients will develop disease in the bone[3], which has been proposed to be a reservoir of malignant cells destined for subsequent metastatic relapse in the bone and visceral organs[4]. Interactions between DTCs and the bone microenvironment lead to pathological bone loss, which can stimulate tumor cell outgrowth. In addition to contributing to morbidity, this 'vicious cycle' also protects tumor cells from the cytotoxic effects of chemotherapy[4]. This protective microenvironment may also create an immune privileged site that prevents immune surveillance[5] and effective immunotherapy. To better target bone metastasis with immunotherapy, we have been examining how T cell immune surveillance and antigen presentation affect metastatic relapse in the bone. In so doing, we discovered that inhibiting colony stimulating factor-1 receptor (CSF1R), which blocks pathologic activities of osteoclasts (OCs), serendipitously also increases antigen presentation by $CD103^+$ dendritic cells (DCs). We hypothesize that thru these activities CSF1R blockade can destroy the "safe harbor" within the BM, leaving DTCs sensitive to attack by immunotherapy. We will test this using animal models and human clinical samples.

A. The tumor immune microenvironment regulates outcomes. The exact composition of immune cells in primary breast tumors is a significant predictor of chemotherapeutic responsiveness and recurrence-free survival[6-9]. In particular, the presence and activation status of tumor specific T lymphocytes is strongly associated with better clinical outcomes[6-9]. Unfortunately for many patients, this is not the dominant immunologic response[7,10]. Instead many patients have significant numbers of tumor-infiltrating innate immune cells including macrophages and immature dendritic cells[11-14]. In addition to instigating immune suppression, these tumor-infiltrating myeloid cells can actively promote resistance to cytotoxic therapies and the survival of DTC at the sites of future metastases [5,7,15-19].

One critical regulator of the pro-tumor activity of myeloid cells is signaling through the receptor tyrosine kinase CSF1R[7,20,21]. In primary mammary tumors, CSF1R signaling is critical for the recruitment, survival, and pro-tumor activities of monocytes and macrophages[7,22-24]. Previously, we demonstrated that inhibition of CSF1R improves the efficacy of neoadjuvant chemotherapy murine mammary tumor models[7,19,25]. While CSF1R inhibition decreased the number of tumor-associated macrophages (TAMs), surprisingly it also restored anti-tumor T cell activity[7,21,26]. This increase in $CD8^+$ cytotoxic T lymphocytes (CTLs) was necessary for CSF1R blockade to enhance chemotherapeutic efficacy and restrain metastasis[7,21]. These provocative findings led our pharmaceutical collaborators (Plexxikon, letter attached) to launch a phase I clinical trial of CSF1R inhibition in combination with chemotherapy in locally recurrent breast cancer (NCT01525602).

B. Targeting CSF1R signaling improves immunotherapeutic response and augments $CD103^+$ DCs. Recently, in pancreatic cancer models, we showed that CSF1R blockade improves immunotherapeutic efficacy in part by increasing antigen presentation by $CD11b^{low}CD103^+$ DCs ([20

and not shown). The enhancement of $CD103^+$ DCs by CSF1R blockade is a novel finding and of critical importance to tumor immunity. This DC subset is critical for sustaining T cell-mediated immune surveillance by antigen cross-presentation to $CD8^+$ T cells in breast tumors[19,27].

2. Keywords:

metastatic breast cancer, immune surveillance, bone, dendritic cells

3. Accomplishments:

3A. Accomplishment on the major goals

(Please note all data figures are attached to the end of this document.)

Aim 1. Major Task 1. Determine the efficacy of CSF1R inhibition in combination with chemotherapy and immunotherapy in mouse models of established metastatic disease: Our assessments of bone metastatic models suggest that neutralization of CSF1R can modestly improve responses to chemo or checkpoint immunotherapy (**Figure 1A-C**). These data while suggesting that neutralizing CSF1R could be an effective approach, but also suggest other factors may compensate for CSF1R neutralization to enforce immune suppression. To further these studies we will investigate these factors as well (see below)

Aim 1. Major Task 2 Determine if CSF1R blockade improves T cell responses in bone metastases by impacting DC subsets. Our investigation in this area found that 1) mature $CD24^+$ and $SIRP\alpha^+$ dendritic cells(DCs) number are dramatically down-regulated in the bone marrow of tumor bearing and bone metastatic mammary tumor models (**Figure 1D-F**). We have also discovered that this down-regulation of DCs is likely due to a change in the differentiation of these cells in the bone marrow. These data were demonstrated using adoptive transfer of DC-progenitor cells in tumor bearing and tumor naïve mice (**Figure 1H**). However, our assessment of neutralization of CSF1R effect on DC subsets determined that CSF1R signaling while increasing DC numbers in the bone marrow of tumor naïve mice, is unable alone to improve $CD24^+$ DC numbers in tumor bearing mice (**Figure 1G**).

Aim 2. Major Task 1

We have collected and analyzed a total of 35 bone-marrow samples and matched blood samples from human breast cancer patients. We have also immune profiled the majority of these samples by high density FACS and compared them to samples from "healthy donors". Our findings are as follows: 1) Several populations of dendritic cells and their pre-cursors are down regulated in cancer bearing patients compared to normal controls (**Figure 2A-B**). 2) The relative number of bone marrow CD141 DCs in the bone marrow of patients correlates with increased response to neo-adjuvant chemotherapy and decreased metastatic recurrence (**Figure 2C**). 3) Circulating numbers of DC progenitors are significantly down-regulated in breast cancer patients blood (**Figure 2D**). Going forward we are continuing to collect data on these cohorts and access the impact on patient outcome.

Aim 2. Major Task 2

This task is slated for year 2

3B. Dissemination of these findings.

These findings were presented as a poster at the Keystone Symposium on "Cancer Pathophysiology: Integrating the Host and Tumor Environments (C3)" in Breckenridge, Colorado March 28th. A publication submission is planned for 2017.

4. Impact
"Nothing to report." This is year 1.

5. Changes/Problems

5A. Changes in approach, additional approaches proposed based on new data:
We are expanding Aim1A and Aim1B to include the neutralization of signaling through CSF2/CSF2R. This was originally proposed as an alternative but likely plays a significant role in DC development and immunotherapy response. We will evaluate this in addition to current CSF1R work.

No changes to human, vertebrate animal, or biohazard compliances.

6. Products
"Nothing to report." This is year 1.

7. Participant and other collaborating organization

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Funding source	DOD, NCI, Departmental funds

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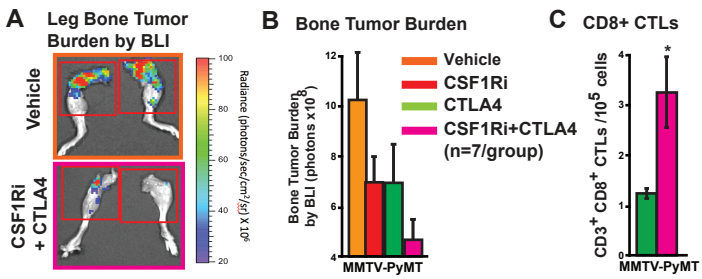
Nearest person months	0.5 month
Contribution to Project	Co-Investigator
Funding source	NCI, DOD

8. Special Interest
"Nothing to report."


A
Leg Bone Tumor Burden by BLI
Vehicle
CSF1Ri + CTLA4
Radiance (photons/sec/cm²/sr) X 10⁶
B
Bone Tumor Burden
Bone Tumor Burden by BLI (photons x10⁸)
Vehicle
CSF1Ri
CTLA4
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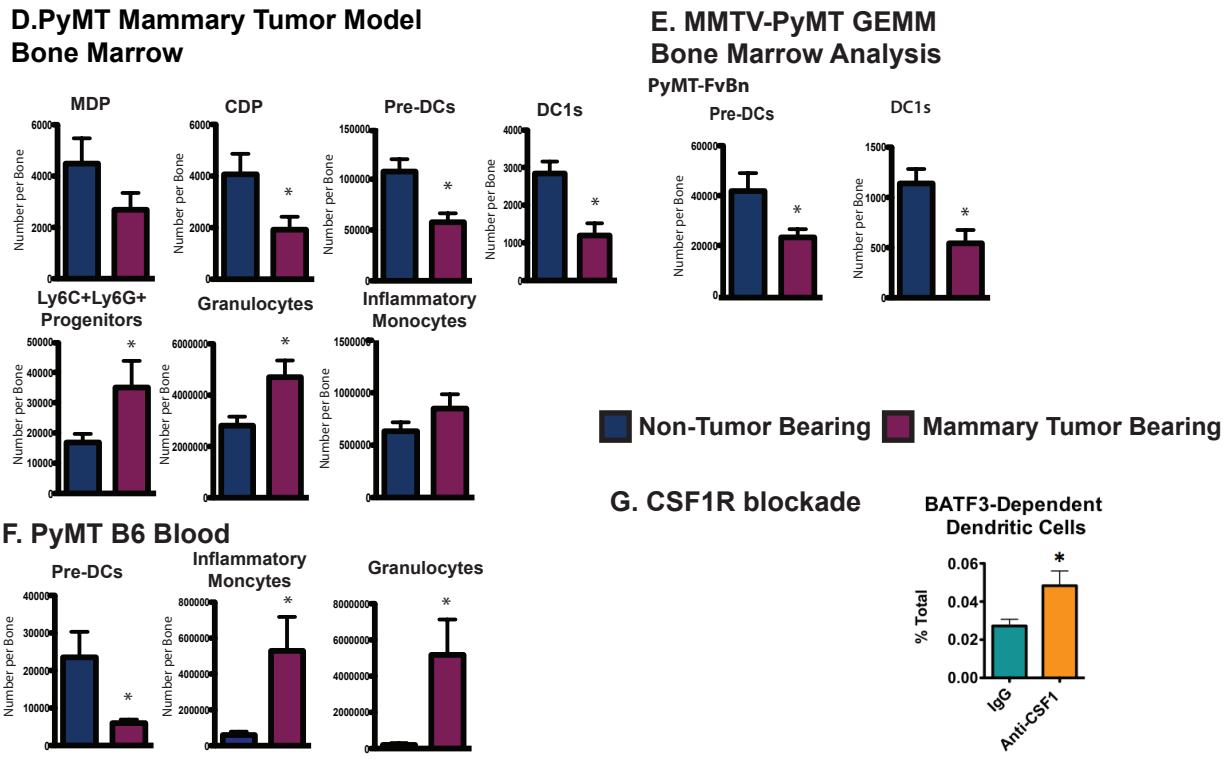
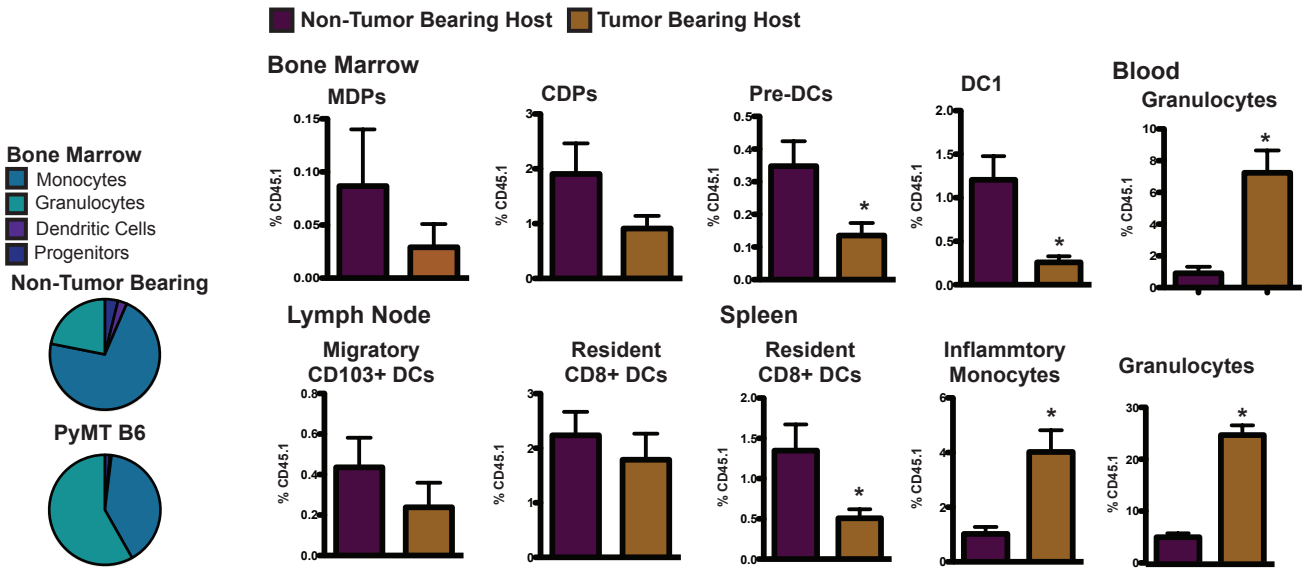
(n=7/group)
MMTV-PyMT
C
CD8+ CTLs
CD3+ CD8+ CTLs /10⁵ cells
MMTV-PyMT


D.PyMT Mammary Tumor Model
Bone Marrow
MDP
CDP
Pre-DCs
DC1s
Ly6C+Ly6G+ Progenitors
Granulocytes
Inflammatory Monocytes
Number per Bone
E. MMTV-PyMT GEMM
Bone Marrow Analysis
PyMT-FvBn
Pre-DCs
DC1s
Non-Tumor Bearing
Mammary Tumor Bearing
F. PyMT B6 Blood
Pre-DCs
Inflammatory Moncytes
Granulocytes
G. CSF1R blockade
BATF3-Dependent Dendritic Cells
% Total
IgG
Anti-CSF1


H. Lin-Sca1-cKit+ Myeloid Progenitors Adoptive Transfer
Non-Tumor Bearing Host
Tumor Bearing Host
Bone Marrow
MDPs
CDPs
Pre-DCs
DC1
Blood
Granulocytes
% CD45.1
Bone Marrow
Monocytes
Granulocytes
Dendritic Cells
Progenitors
Non-Tumor Bearing
PyMT B6
Lymph Node
Migratory CD103+ DCs
Resident CD8+ DCs
Spleen
Resident CD8+ DCs
Inflammtory Monocytes
Granulocytes

DOD-Report 1 Figure 2